Biological Psychiatry: CNNI

Archival Report

Low Smoking Exposure, the Adolescent Brain, and the Modulating Role of *CHRNA5* Polymorphisms

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ABSTRACT

BACKGROUND: Studying the neural consequences of tobacco smoking during adolescence, including those associated with early light use, may help expose the mechanisms that underlie the transition from initial use to nicotine dependence in adulthood. However, only a few studies in adolescents exist, and they include small samples. In addition, the neural mechanism, if one exists, that links nicotinic receptor genes to smoking behavior in adolescents is still unknown.

METHODS: Structural and diffusion tensor magnetic resonance imaging data were acquired from a large sample of 14-year-old adolescents who completed an extensive battery of neuropsychological, clinical, personality, and drug-use assessments. Additional assessments were conducted at 16 years of age.

RESULTS: Exposure to smoking in adolescents, even at low doses, is linked to volume changes in the ventromedial prefrontal cortex and to altered neuronal connectivity in the corpus callosum. The longitudinal analyses strongly suggest that these effects are not preexisting conditions in those who progress to smoking. There was a genetic contribution wherein the volume reduction effects were magnified in smokers who were carriers of the high-risk genotype of the alpha 5 nicotinic receptor subunit gene, rs16969968.

CONCLUSIONS: These findings give insight into a mechanism involving genes, brain structure, and connectivity underlying why some adolescents find nicotine especially addictive.

Keywords: Adolescents, fMRI, Genetics, Gray matter volume, Low smoking exposure, Neuroimaging

https://doi.org/10.1016/j.bpsc.2019.02.006

Smoking is the leading cause of preventable death in the United States and other developed countries. According to the U.S. Centers for Disease Control and Prevention (https://www.cdc.gov/healthcommunication/toolstemplates/entertainmented/tips/GlobalSmoking.html), smoking causes nearly 6 million deaths per year globally; current trends predict this will reach 8 million by 2030. Using the United States as an example, more than 5 million Americans who are under 18 years of age today are expected to die from a smoking-related illness, which is higher than the number of deaths expected to be caused by human immunodeficiency virus, drug misuse, suicide, murder, and motor-vehicle injuries combined. Smoking starts primarily during adolescence, with about 90% of U.S. smokers reporting that they tried smoking before 18 years of age. Every day in the United States alone, 3800 adolescents smoke their first cigarette and 2100 become daily smokers. These statistics are particularly troubling given that early use of cigarettes during adolescence has been associated with heightened risk for later dependence (1,2). Remarkably, even relatively low rates of cigarette consumption during adolescence (e.g., two to four cigarettes per week) increase the risk of becoming nicotine dependent by early adulthood (1,3).

Adolescence is a period of considerable brain development (4,5), and researchers have hypothesized that nicotine use during this critical period produces neurobiological changes that promote tobacco dependence later in life (6). Thus, studying the neural consequences of smoking during adolescence, including those associated with early use, may help reveal the mechanisms that underlie the transition from initial use to nicotine dependence in adulthood. In contrast to the

extensive literature on adult smokers, few studies in adolescents exist, and those available include only small samples.

Nicotine dependence is highly heritable (7), and genome-wide association studies have revealed a reliable association between dependence and single nucleotide polymorphisms (SNPs) at the 15q nicotinic acetylcholine receptor α5-α3-β4 gene cluster (8–10). The most replicated SNPs associated with smoking and lung cancer are the cholinergic receptor nicotinic α3 subunit gene (*CHRNA3*) rs1051730 (11,12) and the α5 subunit gene (*CHRNA5*) rs16969968 (8), both of which convert aspartic acid (G allele) to asparagine (A allele), with A being the risk allele. However, the neural mechanisms, if any exist, that link these SNPs to smoking are poorly understood. One possible mechanism linking the polymorphism to nicotine dependence is reduced dopamine-mediated reward processing owing to A allele nicotinic acetylcholine receptor expression (9). For example, Sherva *et al.* (10) concluded that the A allele was significantly related to enhanced pleasurable responses to a person's first cigarette. Hong *et al.* (13) showed that rs16969968 is associated with a dorsal anterior cingulate–ventral striatal–extended amygdala circuit, in which the risk allele was associated with decreased intrinsic resting functional connectivity strength in smokers and, to a lesser extent, nonsmokers. Further, the connectivity strength of the circuit distinguished smokers from nonsmokers and predicted addiction severity in smokers (13).

In this study, we sought to determine how these genetic predispositions might impact the morphometry of the developing adolescent brain and its relationship to smoking. We were specifically interested in the genetic influence of the *CHRNA3* SNP rs1051730 and the *CHRNA5* SNP rs16969968 on initial cigarette use to illuminate possible pathways that lead to heavier use and dependence. Using multimodal neuroimaging in a large cohort of 14-year-old adolescents from the IMAGEN study (http://www.imagen-europe.com), we first determined gray matter volume (GMV) differences in both early smokers and nonsmokers, and next we determined the influence of the two SNPs of interest. Then, we measured white matter connectivity to determine the anatomical structural connectivity that may have supported these genetic polymorphism findings.

We hypothesize, based on previous neuroimaging studies, that we would find a smoking association with GMV mainly in the prefrontal cortex (PFC) (14–16) and with white matter connectivity mainly in the corpus callosum (17–19). We also hypothesized, based on findings from previous genome-wide association studies (8), that we would observe a small but significant association between *CHRNA5* and smoking behavior.

METHODS AND MATERIALS

Overview of IMAGEN Protocols

Full details of the procedures employed by the IMAGEN study, including details on ethics, recruitment, and standardized instructions for administration of the psychometric and cognitive behavioral measures, are available in the standard operating procedures for the IMAGEN project (https://imagen-europe.com/resources/standard-operating-procedures/).

Participants

Data were acquired from 14-year-old adolescents. After complete description of the study to the participants and their parents and/or guardians, written informed consent was obtained. Individuals who provided assent completed an extensive battery of neuropsychological, clinical, personality, and drug-use assessments online and at the testing centers. Additional assessments were conducted at 16 years of age. Participants were excluded if they had contraindications for magnetic resonance imaging (MRI) (metal or electronic implants or claustrophobia) or problematic medical history (e.g., diabetes, tumors, heart defects), neurological conditions (e.g., epilepsy, head trauma, neurodevelopmental disorders such as attention-deficit/hyperactivity disorder, obsessive-compulsive disorder, depression, or anxiety), or low IQ (<70). The Wechsler Intelligence Scale for Children was used to measure IQ and was administered by experimenters at the study centers. The Vocabulary and Similarities subscales were employed to determine Verbal IQ. The Block Design, Matrix Reasoning, and Digit Span subscales were employed to determine Nonverbal/Performance IQ. A puberty score was calculated using the Pubertal Development Scale (PDS) (20), which consisted of asking adolescents about physical development traits, such as growth in height, body hair, skin changes, and other sex-specific traits (i.e., voice deepening, menstruation). The PDS category scores (answers) are as follows: 1 (No), 2 (Yes [Barely]), 3 (Yes [Definitely]), 4 (Development Completed). Data were normed according to the participant's age.

Smoking Score

A cigarette-smoking score was calculated for the 14-year-old adolescents from the European School Survey Project on Alcohol and Drugs (ESPAD) (21) questionnaire, which asked: "On how many occasions (if any) during your lifetime have you smoked cigarettes?" The ESPAD category scores are as follows: score (number of lifetime occurrences): 0 (0), 1 (1–2), 2 (3–5), 3 (6–9), 4 (10–19), 5 (20–39), 6 (>40). Follow-up smoking scores at 16 years of age were also collected. To account for secondhand smoke exposure, parent smoking scores were calculated using the same ESPAD questionnaire. These scores were not significantly different between smokers and nonsmokers in the sample (1.6 ± 0.5 vs. 1.3 ± 0.8, respectively), with $p = .23$. Similar data using the same scoring system were obtained for alcohol use, where an alcohol score was calculated from a similar ESPAD questionnaire that asked: "On how many occasions (if any) during your lifetime have you had any alcoholic beverage to drink?"

Different numbers of smokers and nonsmokers were available for the neuroimaging and genetic analyses, which are graphically presented in Figure 1. Participant demographics are described in Supplemental Table S1.

Neuroimaging

MRI Acquisition. MRI scanning was performed at the eight IMAGEN assessment sites (London, Nottingham, Dublin, Mannheim, Dresden, Berlin, Hamburg, and Paris) using 3T whole-body MRI systems made by several manufacturers (Siemens Corp., Erlangen, Germany [four sites]; Philips, Best, the Netherlands [two sites]; General Electric Healthcare,

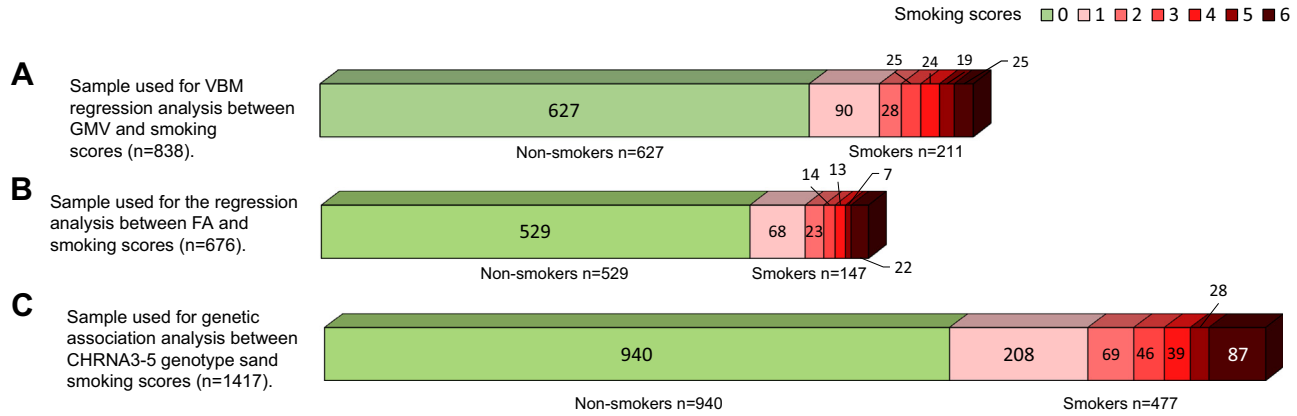

Figure 1. The different participant groups available from the IMAGEN study were used for **(A, B)** the neuroimaging regression analyses and **(C)** the genetic analyses. Nonsmokers are represented with green bars (smoking score = 0). Smokers included in regression and genetic analyses had very low, low, or moderate smoking-exposure levels (smoking scores 1–6). Numbers on bars represent the total number of participants for each smoking score. FA, fractional anisotropy; GMV, gray matter volume; VBM, voxel-based morphometry.

Chicago, IL [one site]; and Bruker, Billerica, MA [one site]). To ensure comparability of MRI data acquired on these different scanners, image-acquisition techniques were implemented using a set of parameters—for example, those directly affecting image contrast or functional MRI preprocessing—that were compatible with all scanners and that were held constant across sites. The full details of the MRI acquisition protocols and quality checks have been described previously, including the extensive period of standardization across MRI scanners (22).

Structural MRI. High-resolution anatomical MRIs were acquired with a three-dimensional T1-weighted magnetization prepared rapid acquisition gradient-echo sequence based on the Alzheimer's Disease Neuroimaging Initiative protocol (http://adni.loni.usc.edu/methods/documents/mri-protocols/).

Diffusion MRI. Diffusion-weighted images were acquired with a single-shot echo-planar imaging sequence with a *b* value of 1300 s/mm^2, an echo time of 104 ms, and a voxel size of 2.4 × 2.4 × 2.4 mm, with 60 slices providing whole-brain coverage.

MRI Data Preprocessing. Preprocessing of the structural T1-weighted data was performed centrally with Statistical Parametric Mapping version 8 (SPM8) (Wellcome Department of Neuroimaging, London, United Kingdom; http://www.fil.ion.ucl.ac.uk/spm/software/spm8/), using standard automated pipelines (22). Structural T1-weighted MRI processing included image segmentation into GM, white matter, and cerebrospinal fluid tissue classes, preceded by an iterative registration to the Montreal Neurological Institute template space, using SPM's optimized normalization routine (23). For voxel-based morphometry (VBM), GM images were smoothed with a full width at half maximum Gaussian kernel of 8 mm, warped to standard Montreal Neurological Institute space, and modulated by multiplying the linear and nonlinear components of the Jacobian determinants generated during spatial normalization. Thus, the dependent measure in subsequent statistical analyses was absolute GMV, facilitating comparisons of volumetric, rather than tissue concentration, differences (24).

The diffusion tensor imaging (DTI) preprocessing was performed with FSL 4.1 (www.fmrib.ox.ac.uk/fsl). The pipeline consisted of the following steps: first, eddy-current and motion correction were applied using an affine registration and the B0 volume of the DTI data as reference. After registration, a brain extraction was applied to remove non-brain tissues, and a first estimation of the diffusion tensor was achieved for each voxel. After B0 unwarping using the magnitude and phase images from the field map acquisition, a second estimation of the diffusion tensor was achieved for each voxel. Finally, fractional anisotropy (FA) maps were generated for each participant.

Genetics

Genotyping and Quality Control. DNA purification and genotyping was performed by the Centre National de Génotypage in Paris. DNA was extracted from whole-blood samples preserved in ethylenediamine tetraacetate Vacutainer tubes (BD, Franklin Lakes, NJ) using Gentra Puregene blood kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Genotype information was collected at 582,892 markers using the Illumina HumanHap 610 and HumanHap 660 Genotyping BeadChips (Illumina, San Diego, CA). The SNPs with call rates of <95%, minor allele frequency <1%, deviation from the Hardy-Weinberg equilibrium ($p \leq .000001$), and nonautosomal SNPs were excluded.

Imputation of Markers Data. Markers data imputation and quality control for ambiguous SNPs, low minor allele frequencies, missingness, and Hardy-Weinberg equilibrium were done with MaCH software (25) following the Enhancing Neuroimaging Genetics through Meta-Analysis 2 guidelines (26). The 1000 Genomes project reference set of markers (http://www.internationalgenome.org/data) was used for the imputation after decreasing the markers from ~41 million to ~13 million relevant genetic variants that were observed more than once in the European populations (26). Both rs16969968 and

rs1051730 data were imputed and had good imputation quality (R^2 imputation quality metric ≥ .87) (Supplemental Table S2).

Missing Demographic Data

Participants with missing data on sex or site were excluded. Missing values on continuous variables were replaced with the mean derived according to the participant's site and sex. Missing values on nominal data were replaced with the mode of that variable for the participant's site and sex. The maximal missing rate for each variable was lower than 10%.

Statistical Analyses

Single SNP Association Analysis. Genotype effects of rs16969968 and rs1051730 on smoking levels were examined using single SNP linear regression in the 1417 participants included in the genetic analysis (described in Supplemental Figure S1C and Supplemental Table S1 [genetic analysis]). The frequencies of the high-risk, intermediate-risk, and normal (no-risk) genotypes for the two SNPs are described in Supplemental Table S2. Four multidimensional scaling components were calculated using a metric model to account for population stratification and were included, in addition to age and sex, as covariates in an additive regression model (genotypes coded as 0, 1, and 2 for the number of risk alleles). These analyses were performed using PLINK version 1.9 (http://zzz.bwh.harvard.edu/plink/plink2.shtml). No other SNPs were investigated in this study.

Brain Voxelwise Analyses. Whole-brain voxelwise multiple regression analyses were performed on GMV and FA maps to identify regions significantly correlated with the smoking score and to test whether effects were observable across the full range of smoking-exposure levels (i.e., scores 0–6). These analyses were performed using the general linear model, performed with the VBM toolbox of SPM8. Age, sex, PDS, handedness, scanner site (dummy coded), performance IQ, verbal IQ, socioeconomic status, and total GMV (only for GMV analyses) were included as nuisance covariates in the design matrix in all analyses. Performance IQ was significantly lower and alcohol use significantly higher in smokers (p values $< .05$) and were included in the subsequent region of interest (ROI)–level analyses. The other variables were not different between the two groups (p values $> .1$).

The resulting set of voxel values constituted a statistical parametric map of the t statistic. We used 3dClustSim, a cluster correction Monte Carlo procedure available in AFNI (http://afni.nimh.nih.gov/), to determine a minimum cluster size that achieves a corrected significance of $p < .05$ with a voxelwise threshold of $p < .001$ and a full width at half maximum spatial blur that is empirically derived from the spatial autocorrelation in the datasets (residuals from the voxelwise statistical analyses). Clusters with a spatial extent threshold >411 voxels were considered significantly related to smoking levels.

ROI-Level Analysis. The mean GMV was extracted with the MarsBaR toolbox (27) from the ROI that the VBM regression analysis revealed to be significantly associated with smoking. GMV values were then included as a dependent variable in a 2 (smoking status) × 3 (genotype) analysis of covariance (ANCOVA) model to test the interaction between smoking and genotype on GMV. In this model, participants were grouped into smokers (score = 1–6) and nonsmokers (score = 0) and then into the three genotypes (AA, GA, and GG). Further, the mean FA was extracted from the ROI obtained from the DTI regression analysis that was significantly associated with smoking and then included as a dependent variable in a similar ANCOVA model to test the interaction between smoking amount and genotype on FA.

RESULTS

Smoking Status and *CHRNA* Genotype Effects on Structural Variations in the Cortex

First, we studied the neuroanatomical correlates of cigarette use in GM density in 211 adolescent smokers from the cohort who had VBM data passing quality control and very low to moderate smoking exposure (smoking scores 1–6) compared with 627 nonsmokers (Figure 1A). A whole-brain VBM

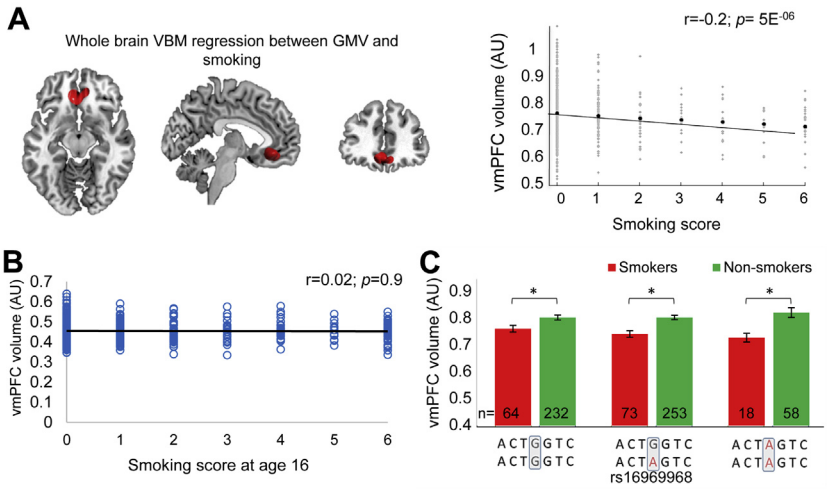

Figure 2. **(A)** Whole-brain rendering of the T maps resulting from the brain voxelwise regression analysis between gray matter volume (GMV) and smoking score. A significant negative correlation (initial threshold $p < .005$; $p < .05$ when corrected for multiple comparisons) was observed in the ventromedial prefrontal cortex (vmPFC). No positive correlations between GMV and smoking score were detected. **(B)** The relationship between the vmPFC volume and future smoking at age 16 in 627 adolescents who were smoking naïve at 14 years of age. No significant correlation was observed, with $r = .02$ and $p = .9$. **(C)** The rs16969968 genotype effects on structural GMV in the vmPFC. A 2 × 3 analysis of covariance indicated that smoking status and the smoking-genotype interaction had significant effects on the vmPFC volume ($p < .0005$ and $p = .026$, respectively), where it was significantly decreased in smokers with the effect being largest in the carriers of the smoking-related high-risk genotype (AA). *Significant difference, $p < .05$. VBM, voxel-based morphometry.

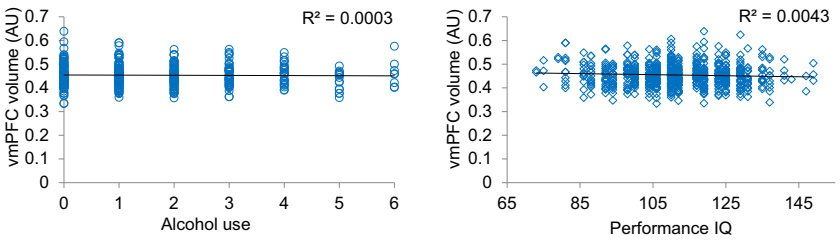

Figure 3. Alcohol use and performance IQ association with ventromedial prefrontal cortex (vmPFC) volume in nonsmokers. No significant correlations were observed in any of the analyses, with $R^2 \leq .0043$ and $p > .1$, suggesting that alcohol use and performance IQ are not correlated with vmPFC volume.

regression analysis showed a significant negative linear relation between GMV and smoking scores ($r_{822} = -.2$, $p = .000005$) in the ventromedial PFC (vmPFC) (cluster size: 499 voxels; cluster peak coordinates: x = 6, y = 30, z = −12) after $p < .05$ clusterwise correction (Figure 2A).

We next replicated the association of the rs16969968 genotype previously reported in smokers (9) in 940 nonsmokers and 477 smokers across all smoking ranges (Figure 1C) ($p = .003$), which explained ~3.5% of smoking behavior variance in the sample (Supplemental Table S2). Analyses of the rs1051730 SNP revealed a similar result to that seen for rs16969968, which is not surprising given their strong linkage disequilibrium ($D' = 1$; $R^2 = .99$; Supplemental Table S2). Assuming that rs16969968 is the functional locus (9), results from rs1051730 are not reported further and subsequent analyses are restricted to rs16969968.

Next, we studied smoking exposure × genotype interaction effects on the lower vmPFC volume derived from the whole-brain regression analysis that provided the vmPFC ROI used for subsequent analyses. A 2 (smoking status) × 3 (genotype) ANCOVA indicated that smoking status (all smoking-exposure levels; $p < .0005$) and the smoking × genotype interaction ($p = .02$) had significant effects on the vmPFC volume (Figure 2C); there was no significant main effect for genotype either in the whole sample or in nonsmokers ($p > .09$). Notably, the ANCOVA showed that the vmPFC volume was significantly lower in smokers in each of the three genotypes, compared with that in nonsmokers ($p = .000012$), with the largest effect in homozygote carriers of the high-risk alleles (AA genotype; $n = 18$ smokers and $n = 58$ nonsmokers). Moreover, smoking levels did not differ significantly among the three genotype groups ($p = .1$).

Alcohol Use and Performance IQ Effects in Smokers

In our sample, alcohol use and performance IQ were significantly associated with smoking (Supplemental Table S1), thus rendering it difficult to attribute the vmPFC anatomical and functional effects to smoking per se. To address this, we identified 341 nonsmokers and assessed the correlations between alcohol use (alcohol scores ranging from 0 to 6 reflecting the same ranges of lifetime use as defined for smoking) and performance IQ with the volumetric measures for the vmPFC. The same covariates were included as above. The results yielded no significant associations with alcohol use ($R^2_{326} < .0005$, $p > .1$) or performance IQ ($R^2_{325} < .01$, $p > .05$) (Figure 3). These results strongly suggest that alcohol use and performance IQ, on their own (i.e., in nonsmokers), do not significantly impact the volumetric effects in the vmPFC and thus are unlikely to be the source of the observed genetic effects in smokers.

Smoking Status Effects on White Matter

Finally, we examined the connectivity of the white matter as a function of smoking. Using the same covariates as in the

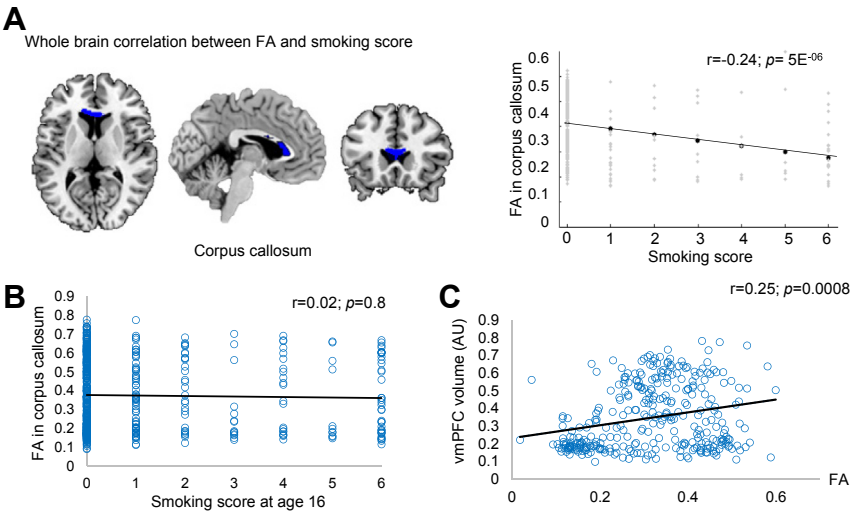

Figure 4. Smoking status effects on white matter integrity. **(A)** Whole-brain rendering of the T map and Pearson's correlation showing significant negative correlation between fractional anisotropy (FA) values in the corpus callosum region of interest and smoking occasions, with $r = -.24$ and $p = .000005$. **(B)** The regression between the ventromedial prefrontal cortex (vmPFC) volume and future smoking at 16 years of age in 531 adolescents who were smoking naïve at 14 years of age revealed no significant correlation relationship, with $r = .02$ and $p = .8$. **(C)** Pearson's correlation test highlighting the significant positive correlation between FA values in the corpus callosum cluster and the vmPFC volume, with $r = .25$ and $p = .00008$. *Significant difference, $p < .05$.

previous analyses (except for total GMV), we performed a whole-brain regression on a sample of 147 smokers and 529 nonsmokers for a total of 676 participants (Figure 1B). The analysis yielded a significant negative correlation ($r_{661} = -.24$, $p = .000005$) between FA and smoking scores in the anterior corpus callosum (359 voxels, x = −15, y = 30, z = 10) after p corrected $< .05$ (Figure 4A), reflecting altered interhemispheric axonal structural properties with smoking and revealing a linear relationship similar to what was observed between the vmPFC volume and smoking. Finally, a 2 × 3 ANCOVA revealed no significant interaction between smoking and genotype on FA extracted from the corpus callosum ROI ($p > 0.1$). While this finding could be explained by the lack of statistical power due to the loss of a significant number of heavier smokers who did not have DTI data, it is also possible that the nicotinic receptor genetic influence was manifest on GMV but not white matter connectivity.

DISCUSSION

In this study, we have shown that exposure to tobacco smoking in adolescents, even at low doses, is linked to a reduction in vmPFC GMV and altered neuronal connectivity in the corpus callosum. Most notably, the regression analyses indicate linear reductions in vmPFC volumes and neuronal connectivity observable in the very-light-smoking group of young adolescents. Finally, there was a small yet interesting genetic contribution wherein the vmPFC volume reduction effects were magnified in smokers who were carriers of the high-risk polymorphisms of the alpha 5 and/or alpha 3 nicotinic receptor subunits. The absence of both main effects of genotype and any genotype effects in nonsmokers indicates a gene × exposure interaction such that the effects of the polymorphisms are evident only if the adolescent is a smoker.

Our structural findings are in line with those of numerous VBM studies reporting negative dose-response correlations between the PFC volume and/or density in general (14–16) or the vmPFC volume in particular (28) and lifetime cigarette usage in adult heavy smokers. Notably, the present results show this relationship in a group of relatively inexperienced adolescent smokers and suggest that the linear decrease is present even at the lightest levels of smoking. These results support a growing literature suggesting that smoking particularly affects the PFC, either from nicotine or from one of the > 4000 chemicals present in tobacco, about 400 of which, including nicotine and carbon monoxide, are known toxins (29).

Despite the converging evidence of apparent brain atrophy in adult moderate and heavy smokers in the PFC (14–16), the dose-response relationship observed in our data is intriguing, as it suggests that just one or two cigarettes can potentially alter adolescent cortex development, an observation that has not been previously reported. An alternative interpretation is that the lower vmPFC GMV preceded smoking initiation and predisposed adolescents toward smoking. To address this possibility, we assessed the relationship between vmPFC volume at 14 years of age and future smoking at 16 years of age in 627 adolescents who were smoking naïve at 14 years of age, using the same covariates included in the previous regression analyses. Within this sample of adolescents, 386 remained never-smokers at 16 years of age, with the remaining 241 showing a similar use distribution in smoking levels at 16 years of age as was observed in the previous analysis of smokers at 14 years of age (see Figure 1). The vmPFC volume derived from the 14-years-of-age regression analysis did not predict future smoking at 16 years of age ($\beta_{611} = .02$, $p = .9$) (Figure 2B), which does not support the hypothesis that the observed volume reduction predisposed individuals toward adolescent use. Rather, this finding is consistent with the interpretation that even extremely low smoking exposure by 14 years of age may influence brain maturation in early adolescence.

The association of the rs16969968 genotype with smoking behavior is consistent with the findings of Hong *et al.* (30), who showed that rs16969968 genotype significantly explains 3.3% and 4.6% of the variance of nicotine addiction severity and cigarettes per day, respectively. The gene × exposure vmPFC effect is consistent with a recent meta-analysis (31) of pharmacological neuroimaging studies that revealed that both cigarette smoking and *CHRNA* agonist administration in adult smokers are associated with lower neural activity in, among other regions, the vmPFC. In line with these findings, another meta-analysis, interrogating the neurobiological targets of pharmacological and cognitive-based treatments for addiction to nicotine, identified similar portions of the vmPFC to have lower activity in smokers (32). Moreover, the rs16969968 × smoking interaction provides evidence that nicotine, rather than the other chemicals in cigarettes, might be the basis of the association between smoking and GMV reductions modulated by the nicotinic acetylcholine receptor system.

The DTI findings are in line with previous data showing that smokers have lower white matter FA in the anterior corpus callosum (17–19), which has been interpreted to indicate possible axonal damage and disrupted myelin integrity in the region (19). Conversely, other studies have reported higher FA (33) or unchanged FA (34) in the corpus callosum of smokers. This discordance could be explained by the fact that the used sample differed by age, size, or smoking exposure. Similar to the GMV findings, FA differences were observed even in very light smokers. To address the possibility that the reduced FA values, like the reduced vmPFC GMV, preceded smoking, we similarly assessed the relationship between anterior corpus callosum FA at 14 years of age and future smoking at 16 years of age in 531 adolescents who were smoking naïve at 14 years of age (348 never-smokers and 183 smokers), using the same covariates as in the previous FA analyses. The anterior corpus callosum FA, derived from the regression analysis, did not predict future smoking ($r_{516} = .02$, $p = .89$) (Figure 4B), which supports the conclusion that the FA reduction did not precede adolescent use but that very low smoking exposure appears to alter adolescent brain development.

Since the anterior corpus callosum connects regions of the PFC with morphologically similar regions in the opposite hemisphere (35), we next asked whether the anterior corpus callosum FA reduction was related to the vmPFC volume reduction of the age 14 smokers. There was a significant positive correlation between the two in smokers and nonsmokers ($r_{615} = .25$, $p < .0001$; Figure 4C) within 630 adolescents (498 nonsmokers and 132 smokers) from the sample used for the whole-brain FA regression analysis who also had

GMV data. Finally, while the absence of interaction between smoking and genotype on FA could be explained by the lack of statistical power owing to the loss of a significant number of heavier smokers who did not have DTI data, it is also possible that the nicotinic receptor genetic influence was manifest on GM volume but not white matter connectivity.

The key limitation of this study is the sample size for the genetic analyses. With 1417 participants for whom genetic data was available (940 nonsmokers, 417 smokers), we are at the lower limit of the genetic-association approaches for estimating contributions of common SNPs to phenotypic variations, especially because individual SNPs explain only small amounts of this variance. The limited sample size also affected the significance values in the brain-genotype interaction analysis where we ended up with a small group (n = 18) of smokers having the double risk allele. The genetic findings must be therefore interpreted in light of the sample-size limitations. Nevertheless, this sample-size limitation must be viewed in the context of the phenotype under study. In fact, the IMAGEN dataset is currently the largest longitudinal brain imaging and genetics study in adolescents worldwide, allowing us to detect unique relationships, even if small, between the brain, genetics, and smoking behavior.

Combined, this study's results indicate a structural and functional basis for dose-response changes in the brain of young adolescent smokers, which may underlie, at least in part, the known *CHRNA* genetic association with smoking. The longitudinal analyses suggest that these effects are not preexisting conditions in those who progress to smoking, but may be, in the case of GMV for example, an initial phase of volume reductions of the PFC in general and the vmPFC in particular that have been observed in adult heavy smokers (14–16,28). Although adolescent experimentation with smoking is common, these results give insight into a mechanism involving genes, brain structure, and brain connectivity underlying why some teens find nicotine especially reinforcing and transition to repeated use leading to increased risk of lifetime dependence.

ACKNOWLEDGMENTS AND DISCLOSURES

This work received support from the National Institute on Drug Abuse and Food and Drug Administration (Grant No. P50DA036114); the National Institute on General Medical Sciences (Grant No. P20GM103644); the European Union-funded FP6 Integrated Project IMAGEN (Reinforcement-related behaviour in normal brain function and psychopathology) (Grant No. LSHM-CT- 2007–037286); the FP7 projects IMAGEMEND (IMAging GEnetics for MENtal Disorders) (Grant No. 602450) and MATRICS (Grant No. 603016); the Innovative Medicine Initiative Project EU-AIMS (Grant No. 115300–2); a Medical Research Council Programme Grant ''Developmental pathways into adolescent substance abuse'' (Grant No. 93558); the Swedish funding agency FORMAS; the Medical Research Council and the Wellcome Trust (Behavioural and Clinical Neuroscience Institute, University of Cambridge); the National Institute for Health Research Biomedical Research Centre at South London and Maudsley National Health Service Foundation Trust and King's College London; the Bundesministerium fur Bildung und Forschung (Grant Nos. 01GS08152, 01EV0711, eMED SysAlc 01ZX1311A, and Forschungsnetz AERIAL); the Deutsche Forschungsgemeinschaft (Reinhart-Koselleck Award No. SP 383/5–1 and Grant Nos. SM 80/7–1, SFB 940/1, and FOR 1617); the French Mission Interministe rielle de Lutte contre la Drogue et la Toxicomanie, the Centre de NeuroImagerie de Recherche, Pr. S. Lehe ricy within the Institut du Cerveau et de la Moelle Epinière; the National Institute of Mental Health (Grant No. MH082116); the National Institutes of Health (Center of Biomedical Research Excellence Award No. P20GM103644 from the National Institute of General Medical Sciences); and the Tobacco Centers of Regulatory Science (Award P50DA036114). HG was supported, in part, by National Institutes of Health Consortium (Grant No. U54 EB020403), which was supported by a cross–National Institutes of Health alliance that funds Big Data to Knowledge Centers of Excellence.

GS is the primary investigator of the IMAGEN Consortium. TB, GB, ALWB, UB, CB, AC, PJC, SD, HF, VF, JG., PG, AH, BI, J-LM, M-LP-M, FN, DPO,L P, TP, MNS, HW, RW, GS and HG acquired and preprocessed the data. BC and HG performed the analyses and wrote the manuscript KJK, SM, PS, ND, KEH, and CO assisted in analytic methods. EAS, STH, AP, and RRA provided expert advice and edited the manuscript.

The authors report no biomedical financial interests or potential conflicts of interest.

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and Nadja Heym (University of Nottingham, Nottingham, United Kingdom); and Zdenka Pausova and Amir Tahmasebi (University of Toronto, Toronto, Ontario, Canada).

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Received Oct 16, 2018; revised Feb 18, 2019; accepted Feb 19, 2019.

Supplementary material cited in this article is available online at https://doi.org/10.1016/j.bpsc.2019.02.006.

REFERENCES

1. Lydon DM, Wilson SJ, Child A, Geier CF (2014): Adolescent brain maturation and smoking: What we know and where we're headed. Neurosci Biobehav Rev 45:323–342.
2. Kendler KS, Myers J, Damaj MI, Chen X (2013): Early smoking onset and risk for subsequent nicotine dependence: A monozygotic co-twin control study. Am J Psychiatry 170:408–413.
3. Riggs NR, Chou C-P, Li C, Pentz MA (2007): Adolescent to emerging adulthood smoking trajectories: When do smoking trajectories diverge, and do they predict early adulthood nicotine dependence? Nicotine Tob Res 9:1147–1154.
4. Paus T, Keshavan M, Giedd JN (2008): Why do many psychiatric disorders emerge during adolescence? Nat Rev Neurosci 9:947–957.
5. Miguel-Hidalgo JJ (2013): Brain structural and functional changes in adolescents with psychiatric disorders. Int J Adolesc Med Health 25:245–256.
6. DeBry SC, Tiffany ST (2008): Tobacco-induced neurotoxicity of adolescent cognitive development (TINACD): A proposed model for the development of impulsivity in nicotine dependence. Nicotine Tob Res 10:11–25.
7. Lessov-Schlaggar CN, Pergadia ML, Khroyan TV, Swan GE (2008): Genetics of nicotine dependence and pharmacotherapy. Biochem Pharmacol 75:178–195.
8. Bierut LJ (2010): Convergence of genetic findings for nicotine dependence and smoking related diseases with chromosome 15q24–25. Trends Pharmacol Sci 31:46–51.
9. Bierut LJ, Stitzel JA, Wang JC, Hinrichs AL, Grucza RA, Xuei X, *et al.* (2008): Variants in nicotinic receptors and risk for nicotine dependence. Am J Psychiatry 165:1163–1171.
10. Sherva R, Wilhelmsen K, Pomerleau CS, Chasse SA, Rice JP, Snedecor SM, *et al.* (2008): Association of a single nucleotide polymorphism in neuronal acetylcholine receptor subunit alpha 5 (CHRNA5) with smoking status and with "pleasurable buzz" during early experimentation with smoking. Addiction 103:1544–1552.
11. Leung T, Bergen A, Munafò MR, De Ruyck K, Selby P, De Luca V (2015): Effect of the rs1051730–rs16969968 variant and smoking cessation treatment: A meta-analysis. Pharmacogenomics 16:713–720.
12. Munafò MR, Johnstone EC, Walther D, Uhl GR, Murphy MFG, Aveyard P (2011): CHRNA3 rs1051730 genotype and short-term smoking cessation. Nicotine Tob Res 13:982–988.
13. Hong LE, Hodgkinson CA, Yang Y, Sampath H, Ross TJ, Buchholz B, *et al.* (2010): A genetically modulated, intrinsic cingulate circuit supports human nicotine addiction. Proc Natl Acad Sci U S A 107:13509–13514.
14. Brody AL, Mandelkern MA, Jarvik ME, Lee GS, Smith EC, Huang JC, *et al.* (2004): Differences between smokers and nonsmokers in regional gray matter volumes and densities. Biol Psychiatry 55:77–84.
15. Gallinat J, Meisenzahl E, Jacobsen LK, Kalus P, Bierbrauer J, Kienast T, *et al.* (2006): Smoking and structural brain deficits: A volumetric MR investigation. Eur J Neurosci 24:1744–1750.
16. Zhang X, Salmeron BJ, Ross TJ, Geng X, Yang Y, Stein EA (2011): Factors underlying prefrontal and insula structural alterations in smokers. Neuroimage 54:42–48.
17. Lin F, Wu G, Zhu L, Lei H (2013): Heavy smokers show abnormal microstructural integrity in the anterior corpus callosum: A diffusion tensor imaging study with tract-based spatial statistics. Drug Alcohol Depend 129:82–87.
18. Savjani RR, Velasquez KM, Thompson-Lake DGY, Baldwin PR, Eagleman DM, De La Garza R, *et al.* (2014): Characterizing white matter changes in cigarette smokers via diffusion tensor imaging. Drug Alcohol Depend 145:134–142.
19. Viswanath H, Velasquez KM, Thompson-Lake DGY, Savjani R, Carter AQ, Eagleman D, *et al.* (2015): Alterations in interhemispheric functional and anatomical connectivity are associated with tobacco smoking in humans. Front Hum Neurosci 9:116.
20. Petersen AC, Crockett L, Richards M, Boxer A (1988): A self-report measure of pubertal status: Reliability, validity, and initial norms. J Youth Adolesc 17:117–133.
21. Hibell B, Andersson B, Bjarnason T, Kokkevi A, Morgan M, Narsuk A (1997): The 1995 ESPAD report: Alcohol and other drug use among students in 26 European countries. Stockholm, Sweden: Modin-Tryck AB.
22. Schumann G, Loth E, Banaschewski T, Barbot A, Barker G, Büchel C, *et al.* (2010): The IMAGEN Study: Reinforcement-related behaviour in normal brain function and psychopathology. Mol Psychiatry 15:1128–1139.
23. Ashburner J, Friston KJ (2005): Unified segmentation. Neuroimage 26:839–851.
24. Ashburner J, Friston KJ (2000): Voxel-based morphometry—The methods. Neuroimage 11:805–821.
25. Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR (2010): MaCH: Using sequence and genotype data to estimate haplotypes and unobserved genotypes. Genet Epidemiol 34:816–834.
26. Enhancing Neuroimaging Genetics through Meta-Analysis 2 Genetics Support Team. ENIGMA2 1KGP Cookbook (v3) [Online]. 2013. Available from: http://enigma.ini.usc.edu/wp-content/uploads/2012/07/ENIGMA2_1KGP_cookbook_v3.pdf. Accessed February 9, 2018.
27. Brett M, Anton J-L, Valabregue R, Poline J-B (2002): Region of interest analysis using an SPM toolbox [abstract]. Presented at the 8th International Conference on Functional Mapping of the Human Brain, June 2–6, 2002, Sendai, Japan; abstract 497.
28. Fritz H-C, Wittfeld K, Schmidt CO, Domin M, Grabe HJ, Hegenscheid K, *et al.* (2014): Current smoking and reduced gray matter volume—A voxel-based morphometry study. Neuropsychopharmacology 39:2594–2600.
29. Swan GE, Lessov-Schlaggar CN (2007): The effects of tobacco smoke and nicotine on cognition and the brain. Neuropsychol Rev 17:259–273.
30. Hong LE, Yang X, Wonodi I, Hodgkinson CA, Goldman D, Stine OC, *et al.* (2011): A CHRNA5 Allele Related to Nicotine Addiction and Schizophrenia. Genes Brain Behav 10:530–535.
31. Sutherland MT, Ray KL, Riedel MC, Yanes JA, Stein EA, Laird AR (2015): Neurobiological impact of nicotinic acetylcholine receptor agonists: An activation likelihood estimation meta-analysis of pharmacologic neuroimaging studies. Biol Psychiatry 78:711–720.
32. Konova AB, Moeller SJ, Goldstein RZ (2013): Common and distinct neural targets of treatment: Changing brain function in substance addiction. Neurosci Biobehav Rev 37:2806–2817.
33. Hudkins M, O'Neill J, Tobias MC, Bartzokis G, London ED (2012): Cigarette smoking and white matter microstructure. Psychopharmacology (Berl) 221:285–295.
34. Paul RH, Grieve SM, Niaura R, David SP, Laidlaw DH, Cohen R, *et al.* (2008): Chronic cigarette smoking and the microstructural integrity of white matter in healthy adults: A diffusion tensor imaging study. Nicotine Tob Res 10:137–147.
35. Park H-J, Kim JJ, Lee S-K, Seok JH, Chun J, Kim DI, *et al.* (2008): Corpus callosal connection mapping using cortical gray matter parcellation and DT-MRI. Hum Brain Mapp 29:503–516.